



KAROLINSKA INSTITUTET
a medical university

EXHIBIT 1

Department of CLINICAL IMMUNOLOGY

Laboratory Journal No.

Name: ~~XXXXXXXXXX~~
JANS + LIND

Group:

Date: [REDACTED] from to



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Study

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27

Assembly of a synthetic gene coding for
Fcd 1 chain 1 using Tag

PCR

Oligos 132, 133, 6T, 155 10 μ M1 μ l of each oligo 132-1331 μ l of NTP 10 mM0.5 μ l Tag1 μ l 10x Tag-buffer35 μ l H₂O10 μ l

→ PCR Eppendorf program HAN51

94°C 1 min

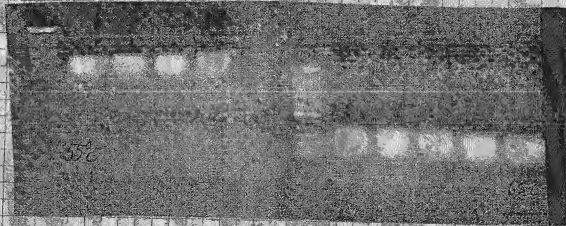
annealing 63-65°C 15 min (grad)

elongation 68°C 2 min

30 cycles ending by 10 min elongation

74°C

Result A strong band around 300 (exp. ~ 200)



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248910

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Study

Assembly of chain 2. Rd. di.

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primers 127-131 + 138

Using 3 different DNA polymerases

Tag
ppa

Method: assembly chain 1st

Ampl. tag

expected band
at 394



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Expression and purification of Fcd1 chain 1 and chain 2.

Fcd1 chain 1 (clone 42) and Fcd1 chain 2 clone 29 was ligated into pET 20b and electroporated into BL-21 DE3 plys after having been cut from pTZ-Blue containing the correct sequence (see binder HCL Fcd1). Sequencing of pET 42/pET 29 was done according to standard protocol (APBT) and the result can be seen on the opposite note.

Both Fcd1 chain 1 (Fcd1:1) and Fcd1 chain 2 (Fcd1:2) was expressed according to standard protocol and purified on a HiTrap (chelex) column loaded with NiSO_4 .

Chain 1 was soluble, after ultra sonication chain 1 was found in 20mM Tris-HCl pH fraction, while chain 2 was found in the inclusion bodies after "washing" with 2M urea buffer + 20mM Tris-HCl pH 8.0. The inclusion bodies were solubilized in 6M Guanidine, transferred to 6M Urea buffer (20mM Tris-HCl pH 8.0 + 0.5M NaCl) via 6th HiTrap. Purification was done on HPLC.

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Erstgenannte: Albert D.

[illegible]

2314

```

SOURCE: D:\C:\WINDOWS\SYSTEM32\cmd.exe
NEW MACS: fileshare
Received by: Protocol
Source file: C:\Program Files\Internet Explorer\iexplore.exe
User name: user
Current version: Microsoft SQL V.0.0.0
Title: 0
PID: 0
405ms
426ms
Exp Time: 0.0015
Interval between calls: 20.000000
Mix: 785
Mix HW: 0.0000
Mix SW (seconds): 0.0000
Mix SW per call: 0.000000
Comments:

```

[illegible]

	(20)	(21)	(22)	(23)	(24)	(25)	(26)
log	0.24	0.25	0.26	0.27	0.28	0.29	0.30
log	0.24	0.25	0.26	0.27	0.28	0.29	0.30
log	0.24	0.25	0.26	0.27	0.28	0.29	0.30
log	0.24	0.25	0.26	0.27	0.28	0.29	0.30

[illegible]

Containing: A = 12 pg/week
B = 9 pg/week
C = 23 pg/week
D = 1 pg/week



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ELISA conditions

Coating of Ed.C1 and Ed.C2 ^(100 µl/well) over the weekend in +4°C serum 4 times with "Tris coating". Patient's serum was added, 100 µl/well and incubated at +4°C o.n. Wash 4 times (Wallac ELISA-washer) with "Tris coating" and 100 µl ^{of} Rabbit human anti IgE dil. 1:500 times in "Van buffer". Incubation 2 hours in RT on shaker. Wash 4x "Tris coating" and add 100 µl/well of Goat anti-rabbit-ALP conjugated (DAKO) for 1h. Wash 4 times and add substrate 3 tablets/15 µl of Van buffer.

The result was read in ELISA reader after 45 min at 405 nm

Result: 2.5 µg/ml serum to be an adequate coating concentration for both chains and chain 2. Mixing of the two chains can be done with coating concentrations 2.5 + 2.5 µg/ml

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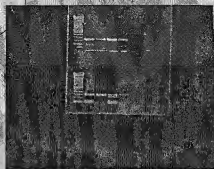
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12 16 20 26

↓ ↓ ↓



2a 36 42 46

Putting chain 1 and 2 (1+2)
together (001121)

template 1a 6ul
template clone 2g
1:10, 1:100, 1:1000

1ul
0.5ul primer 176
2ul 183
2ul dNTP
3ul 10x buff.
6ul pfu
14.5ul H₂O

30ul



↑ ↑ ↑
1:10 1:100 1:1000

Result: One band
at ~500bp which
could be clone 1+2

The bands are cut
out and purified
on Qiaspin

ligated with "perfectly
cloning kit". 10 colonies
are picked for miniprep
and possibly sequencing

- (1a) 1ul template clone 11.42 (1:1000)
2ul primer 176
2ul 183
2ul 10x buff
1ul pfu
2ul dNTP (10mM)
10ul H₂O
20ul

- (1b) Some overhang
primer Tag polymerase

- (2a) 1ul templ. (1:1000) clone 2
1ul templ. 183
2ul primers 176, 183, 185
1 overhang from 1a

- (2b) Some 2a new Tag

- (3a) 1ul template clone 2 (1:1000)
2ul 183
2ul 185
1 overhang from 1a

- (3b) Some 3a new Tag

- (4a) 1ul templ. 1
1ul templ. 2
2ul primer 180
1 overhang from 3a

- (4b) Some 4a new Tag

AmpliTaq Gold
250 Units, 50ul
Store at -20°C

Applied Biosystems
A05912



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Study

Linking of (chain 1 and chain 2 (scamless))
with PCR. Del d1 and Del d2

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Aim. The aim of this experiment is to join the two chains of Fel d1 into one construct by PCR

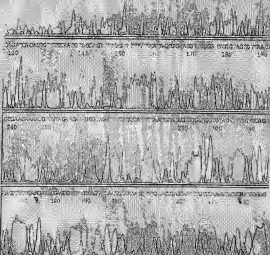
Study outline The two sequenced chains of the major ~~chain~~^{allergen} of cat Fel d1 (chain 1, clone 42) and chain 2, clone 29) is joined with PCR in two steps as outlined below. In



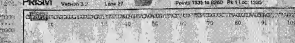
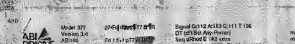
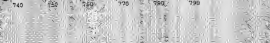
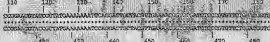
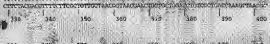
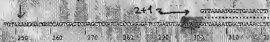
Result (see opposite side)

Good bands of expected size was seen for both chain 1 and chain 2. But (2a) + (2b) as well as (1a) and (1b) did not work. I will continue by adding (1a) to ~~template~~^{template} 29 and do PCR. (Chain 1+2)





Td1 (2+1) PT7 d. 1 R 001205 (t. 7 term.)





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Study

Project no.

Sequencing of 4 clones of each 1+2 and 2+1

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1+2 Clones sig no
 ↓ ↓
 Clone 1 = ①
 2 = ②
 5 = ⑤
 9 = ④

2+1

Clone 1 = ⑤
 6 = ⑥
 7 = ⑦
 9 = ⑧

⑤ Oocyte red - green
 ⑥ whole green
 ⑦ OK, sea (green)

4.8 µl reaction
 1.2 µl primers
 4 µl mix 80 cycler
 10 µl

+ 50 µl oil and

PCR for Partition Filter

98°C 30"
 50°C 15"
 60°C 4"

} 25 cycles

Unfortunately there was a scheduled power failure
 and the PCR-run was interrupted. ^{However} Assam 7 more
 cycles which is done

samples are loaded on lanes 20 - 33 on ABI seq

and named. For 1+2 pT7 clone 4F

- " - 4R
 - " - 5F
 - " - 5R
 etc.

Cutting of clone 5 (1+2)

and clone 1 (2+1)

with Nde I and Xho I

for ligations into pET 28b

20 µl plasmid mix prep (pT7+Blue)

2.0 µl DNA ligation

0.3 µl BSA

0.7 µl Nde I

0.7 µl Xho I

Ligate 37°C shake

for 2h.

→ Please
proof

clone 1 (2+1)
 clone 5 (1+2)

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Study

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Ligation of Pcl 1, clone 1 (2cl) and clone 5 (1cl)
into pET 20b+ and electroporation into BL21 plys

The fragments from Pcl 1 (2cl) clone 1 and clone 5 (1cl)
were digested purified from 1% agarose gel (2x8931)
A cleaned (Nde/Xho) pET 20b+ vector was used
to ligate the fragments

Conculation

(2 μ l vector fragments)
10 μ l vector
1.5 μ l 10x ATP
1.8 μ l 10x ligation buffer
1.7 μ l 1% ligase
1.3 μ l

Ligate +16°C o.n.

The ligate mix was electroporated into 50 μ l BL21-
- plys electrocompetent cells. 1 μ l ligate mix
was added to thawed cells (on ice). Electroporation
according to standard protocol. Growth on SOC
medium for 60' 37°C shaker (300 rpm) and
plated on Amp/CAN plates. One colony from
each plate was picked and grown on LB Amp
can medium, miniprep (G. gen)
and 25 μ l of the (50 μ l) prep was
cut with Nde and Xho. Result ~ 500bp -
Both clones contain the insert!!

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Purification of Fd 1 (1+2) clone 5 and
Fd1 (2+1) clone 1 over Ni²⁺ chelate Hi-Trap

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1 liter of Fd1 (1+2) and (2+1) ^(in pet 200 in B2-2, P149) Vesp. 6 medium (CAM, Am) was grown to 0.6 (600nm) and induced with 0.4 mM IPTG. (see 248932)

Purification according to protocol. Both proteins were expressed as inclusion bodies and purified accordingly. Purification on FPLC is follow. After adsorption onto Hi-column with 6M Gna and wash also with 6 M Gna. The column is stuck to FPLC.

Program:

D conc % 0.0

A = 6 M Urea

0 ml/min 3.0 ml/min

B = 20 mM Imidazole

0 0.25 ml/min

C = 500 mM Imidazole

0 port Set 6

20 conc % B 0

80 conc % B 1.00

100 conc % B 1.00

125 conc % B 0

125 port set 6.0



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